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1 **Recovery of gastric evacuation rate in Atlantic cod *Gadus morhua* L surgically**
2 **implanted with a dummy telemetry device**

3
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9
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11
12 *Short title* Gastric evacuation in surgically implanted cod

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18 **Abstract**

19 The current study investigated how the gastric evacuation rate (GER) was affected after surgically
20 introducing dummies of a blood-flow biotelemetry system into the abdominal cavity of Atlantic
21 cod *Gadus morhua*. Gastric evacuation experiments were performed two and ten days post
22 surgery on surgically implanted and control *G. morhua* force-fed sandeel *Ammodytes tobianus*.
23 The results were compared with previously obtained estimates from unstressed conspecifics
24 voluntarily feeding on a similar diet. After two days, GER was significantly lower in the group of
25 fish with the dummy implants compared to the control group, but following ten days of recovery
26 no significant difference was seen between the two groups. The difference between implanted
27 and control fish observed 2 days post surgery may have arrived either from surgery, post-surgical
28 stress and/or the presence of the implant. The conclusion is that 10 days of postsurgical recovery
29 will stabilize GER in *G. morhua*, thus indicating that at this point the implant *per se* did not affect
30 GER. Both the fish with surgical implants and controls in this study evacuated their stomachs much
31 slower and with much higher inter-individual variation compared to *G. morhua* feeding voluntarily
32 on similar prey items.¹ The lower GER and higher inter-individual variation for force-fed fish
33 indicates that handling, anaesthetization and force-feeding impair GER and that individual fish
34 respond differently to the suppressing effects.

35

36 **Keywords** Post-surgical recovery, gastric evacuation, *G. morhua*, implant, biotelemetry

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41 Invasive procedures are often necessary for the physiologist to understand the mechanisms
42 behind various physiological parameters. In fish, measurements of cardiovascular parameters such
43 as blood flow and blood pressure involve handling, anaesthesia and surgical implantation of
44 catheters and flow probes in and around vessels and sutures to close the incisions. In traditional
45 laboratory experiments, standard bench top blood pressure and blood flow measuring units are
46 used, which means that the animals are 'hardwired' to the equipment during the experiments.
47 Catheters and leads from flow probes penetrate the body wall and increase the risk of infection.
48 Furthermore, to prevent tangling and ripping of wires the animals are most often confined in small
49 containers, which severely limits the range over which they can move with the risk of introducing
50 confinement stress. The animals are usually left for 18 to 48 hr before experimentation is initiated,
51 the main reason for such short periods being the high risk of infection after an extensive surgery.²
52 In cases where catheters are used, limits on the time where these will remain open may also be
53 decisive. However, unless appropriate recovery time is ensured following surgical procedures and
54 instrumentation major concerns are the validity of data and ethical considerations. In a study on
55 rainbow trout *Oncorhynchus mykiss* using bioelectric potential recordings to measure heart rate,
56 fH, (i.e. a non-invasive method) the fish were handled and then left to recover for at least 3 days
57 where after their basal heart rates were much lower than those presented in previous studies
58 using traditional methods involving surgical procedures.³ A lower heart rate usually is indicative of
59 lower stress levels pointing to that these animals were not as stressed due to confinement and the
60 use of 'hardwired' techniques. Obviously, any misinterpretation of data may have important
61 consequences for our understanding of the physiology of fishes and researchers should be
62 encouraged to seek refinements of techniques to ensure more valid results as well as better
63 animal welfare.

64 The rapid development of telemetric devices opens new research areas that have not been
65 possible with hardwired animals and during the last few decades there have been great
66 advancements made in applications of biotelemetry in fish physiology. Biotelemetry allows data
67 collection from specimens that move around freely and can behave in a more natural way. In the
68 telemetrically instrumented animal the risk of infections is lower since there are no skin-
69 penetrating wires. Furthermore long term recovery- and recording periods are possible reducing
70 stress from handling, making this an attractive alternative. Altogether, this provides a number of
71 welfare advantages and the opportunity to obtain higher quality of the measured variables and
72 more reliable routine values. A recent study on white sturgeon (*Acipenser transmontanus*) showed
73 that routine heart rate of freely moving biotelemetric instrumented fish was lower compared with
74 hardwired, confined conspecifics as well as with previous values reported for white sturgeon.^{4, 5}
75 The downside is that the animals are carrying the entire recording/transmitting unit, increasing
76 their body mass and possibly impairing the capacity to generate thrust by altering the ability to
77 bend the body. It may furthermore interfere with the internal organs if placed in the body cavity.
78 Nevertheless, a fully implantable system maximizes the likelihood that the studied fish will be
79 treated normally by surrounding fish⁶ and minimizes the risk of infection and expulsion.⁷ Even the
80 presence of a small, protruding antenna has been shown to cause adverse tissue reaction at the
81 antenna exit^{8, 9} and to elicit aggressive attacks from other individuals of Atlantic salmon smolts.⁶
82 We are looking to use a fully implantable biotelemetry system^{5,10} to study blood flow distribution
83 in Atlantic cod *Gadus morhua* L. voluntarily feeding and moving between different habitats. Before
84 employment of such a system it is, however, imperative to evaluate appropriate post-surgery
85 recovery time and possible adverse effect of the telemetric implant. Using a dummy version of the
86 dual blood flow biotelemetric system used by Gräns and co-workers,^{5, 10} the aim of the present

87 study was to examine the effects on the gastric evacuation rate (GER) of handling, surgery and
88 instrumentation of *G. morhua* with a dual channel blood flow telemetric system following short
89 term (two days post surgery) as well as prolonged (ten days post surgery) recovery. Both the
90 surgically implanted and control fish were force-fed in order to standardize the amount of food
91 and the time of feeding. The ability to evacuate the force-fed meal was determined and compared
92 with estimates obtained from unstressed conspecifics that fed voluntarily.

93 **Materials and methods**

94 **Fish**

95 Wild *G. morhua* ranging in total body mass from 290 to 815 g were caught in late 2009 by fyke net
96 in the vicinity of the Marine Biological Laboratory in the northern part of Øresund, Denmark. The
97 fish were transferred to the laboratory where they were kept in 9-10 °C re-circulated, aerated
98 seawater. The tank was covered by tarpaulin to avoid unnecessary disturbance of the fish.
99 Light/dark conditions were 16h: 8h. All fish were acclimated to laboratory conditions for a
100 minimum of 12 weeks before experiments were initiated and fed *ad libitum* three times a week
101 with chopped herring *Clupea harengus* L. or Raitt's sandeel *Ammodytes marinus* Raitt. Before the
102 experiment the fish were starved for five days to ensure that their stomachs were empty.

103

104 **Preoperative care**

105 Individual *G. morhua* was anaesthetized in 15 l of water containing 0.15 g l⁻¹ 3-aminobenzoic acid
106 ethyl ester (MS-222) until gill ventilation ceased. Each fish was weighed and length measured
107 before it was positioned with the ventral side facing up on an operating table covered with wet
108 sponges. Anaesthesia was maintained during the surgery by pumping oxygenated sea water

109 containing MS-222 (0.075 g l^{-1}) over the gills. All surgical instruments, the dummy implant and
110 associated leads were sterilized by the use of a cold sterilant (Cidex, Johnson & Johnson Company,
111 USA).

112

113 **Dummy implant**

114 Our long-term goal is to implement the fully implantable biotelemetry system used by Gräns et
115 al.^{5, 10} This system weighs 35g (total mass of implant and battery) in air and we aim for a
116 transmitter:fish mass ratio of 3-4%. Consequently, the dummy implant was constructed so that its
117 total mass equaled 19 g in air, thus, corresponding to an average of 3.7 (0.3) % of the mean body
118 mass of the present experimental *G. morhua*. It was made of silicon-coated stainless steel (L = 35
119 mm, H = 5 mm, W = 16 mm) with an attached silicon-coated dummy battery made of plastic tubes
120 (L = 18 mm, D = 11 mm) and two silicon leads with a probe cuff each, and the implant fitted easily
121 within the abdominal cavity of the fish.

122

123 **Surgery**

124 The splanchnic circulation in *G. morhua* is derived from the celiacomesenteric artery (CoMe). The
125 CoMe is the largest single branch of the dorsal aorta and branches to form the celiac artery and
126 mesenteric artery. The celiac artery supplies parts of the cardiac stomach, the pyloric stomach, the
127 proximal intestine and parts of the distal intestine, while the mesenteric artery supplies the
128 remainder of the gastrointestinal system, the gallbladder and the spleen.¹¹ The surgical
129 procedures were performed on 10 fish [body mass 550 (144) g and length 38.7 (3.2) cm] and done
130 in accordance with the guidelines described in permission 2010/561-1812 from the Danish
131 Ministry of Justice. For placement of the first dummy probe, a 2-cm-long incision was made

132 alongside the basibranchiale bone left of the midline, and the liver carefully retracted, where after
133 the CoMe artery was exposed and cleared. The dummy implant and battery were then carefully
134 placed in the abdominal cavity and the retracted organs restored to their places of origin.
135 Furthermore, an individual PIT tag (12×2 mm, ISO standard 11784/11785, FDX-B) was placed in the
136 body cavity for later identification of the fish. To access the ventral aorta for placing the second
137 dummy probe, a 0.5-cm ventral incision was made posterior to the gill juncture and carefully,
138 without disrupting the pericardium or damaging any vessels, the dermal and sub-dermal
139 musculature and connective tissue were separated using blunt dissection tools to expose the
140 ventral aorta. The probe was tunneled under the skin from the abdominal cavity to the incision
141 above the ventral aorta (see Figure 1 for schematic overview of positioning of the system). The
142 wires from the probes were anchored with a single stitch of 3/0 silk suture in the sub-dermal
143 muscle tissue and the two incisions were closed using sterile monofilament prolene 3-0 suture.
144 Subsequently the cod was given a subcutaneous injection of 0.4 mg/kg [1 M] butorphanol
145 (Torbugesic , Fort Dodge, Iowa, USA) for postoperative pain relief in addition to an injection with
146 10 mg/kg [1 M] Enrofloxacin (Baytrilo, Bayer, USA) antibiotics, to minimize the risk of infection.
147 Finally, to reduce incidence of oomycete infections povidone iodine powder was applied to the
148 closed incision before returning the fish to recovery water. The surgical procedure including
149 anaesthesia and awakening took approximately 20 min for each fish and when ventilation and
150 locomotion had been reestablished, the fish were returned to their home tank. All but one fish
151 survived the surgery.

152 The control fish [n=8, body mass 518 (155) g and length 37.1 (2.7) cm] were anaesthetized as
153 described above, and subsequently weighed, length measured and individually PIT tagged. The

154 tags were placed in the abdominal cavity, using a syringe implanter, taking care not to harm any
155 organs. The control fish were likewise left to recover before being returned to their home tank.

156

157 **Food**

158 *A. marinus* constitutes a natural and large part of the diet of wild *G. morhua* and one specimen per
159 cod was used as the experimental meal. To minimize variation in size and condition, specimens of
160 *A. marinus* from a single commercial batch (TripleNine, Hvide Sande, Denmark) were used for
161 force-feeding. The energy density of these prey fish was determined by bomb calorimetry [IKA C-
162 7000 bomb calorimeter (www.ika.net)] on a representative sample after drying it at 60° C until
163 constant mass according to the procedure described in Pedersen & Hislop.¹²

164

165 **Gastric evacuation experiments**

166 The first experiment was initiated 48 hr post surgery. On the day of experiment, *A. marinus* were
167 quickly thawed in running water, and those of similar total lengths were selected. These were
168 subsequently dabbed dry with paper towel and weighed, and individuals within a mass range of
169 9.4-15.7 g were chosen (corresponding to 2-3% of the body mass of the *G. morhua*). This food
170 ration was chosen from the largest daily feeding rates observed in wild *G. morhua*.¹³ In random
171 order individual cod were anaesthetized by placing them in 15 l of water containing 0.07 g l⁻¹ MS-
172 222. They were then force-fed by gently pushing a whole *A. marinus*, bend on the middle, through
173 the esophagus and into the stomach. Immediately thereafter the fish were returned first to
174 recovery water and subsequently to their home tank. The exact time of force-feeding was noted
175 for each fish, as was the length and mass of the prey.

176 Stomach contents were recovered 22 to 26 hr post force-feeding by stomach flushing following
177 anaesthetization (0.07 g l⁻¹ MS-222). The stomach content from each fish was collected on a filter
178 with mesh-size 200 µm, and gently patted with a moist paper towel and weighed. The exact time
179 for recovery of stomach content was noted for each cod.

180 The fish were subsequently left to recover for 7 days in their home tank where after the above
181 described protocol was repeated in a second trial.

182

183 **Calculations and statistical analyses**

184 In accordance with the cylinder model of Andersen & Beyer,¹⁴ the gastric evacuation rate of
185 stomach contents in *G. morhua* (as well as in a variety of other piscivorous fishes) can be described
186 independently of meal size by the current mass of stomach contents S_t (g) together with the rate
187 parameter ρ :

188

$$189 \quad \frac{dS_t}{dt} = -\rho\sqrt{S_t} \quad (1)$$

190 Integrated from time 0 of force-feeding to time t (h) of recovery of stomach contents, the solution
191 to equation (1) is *G. morhua* evacuating its stomach contents according to the relationship

192

$$193 \quad \sqrt{S_t} = \sqrt{S_0} - \frac{1}{2}\rho t; \quad 0 \leq t \leq t_{end} = 2\rho^{-1}\sqrt{S_0} \quad (2)$$

194

195 where t_{end} is the time for complete evacuation of the prey.

196

197 The ability to evacuate the stomach contents is then described by ρ , which can be calculated for
 198 each individual *G. morhua* by reorganization of equation (2):

199

$$200 \quad \rho = 2(\sqrt{S_0} - \sqrt{S_t})t^{-1} \quad (3)$$

201

202 The rate parameter ρ depends on predator (total) length L (cm), temperature T (°C) and prey
 203 energy density E (kJ g⁻¹). The effects of these variables were estimated by Andersen (2001) for
 204 different gadoids fed fish prey, and the following relationship was obtained:

205

$$206 \quad \rho = \rho_{LTE} L^{1.44} e^{0.078T} E^{-0.86} \quad (4)$$

207

208 The basic rate parameter ρ_{LTE} specifies the general ability of the predator to digest and evacuate a
 209 specific prey type from the stomach (or reversed: it indicates the resistance of the specific prey to
 210 the digestive processes in the stomach). The value 0.00142 ± 0.00011 (estimate \pm S.D.) of ρ_{LTE} was
 211 obtained from an experiment by Andersen¹ on *G. morhua* that fed voluntarily on lesser sandeel
 212 *Ammodytes tobianus* L. The temperature (10.3° C) and mean body size of *G. morhua* (41 cm; 664
 213 g) were similar to those of the present study. This value of ρ_{LTE} was used to decide the evacuation
 214 time t of c. 24 h employed in this study, which is shorter than t_{end} and yet long enough to ensure
 215 that a significant part of the meal was evacuated from the stomach prior to recovery of the
 216 remains.

217 *G. morhua* used in the present study varied to some extent in size (Table 1). ρ_{LTE} was therefore
 218 used to represent the ability of individual *G. morhua* to evacuate their force-fed meals of *A.*
 219 *marinus*. By use of this parameter, the results could further be compared directly with the above

220 estimate obtained from unstressed *G. morhua* that fed voluntarily. Combining equations (3) and
 221 (4), the value of ρ_{LTE} for each individual *G. morhua* was so calculated by

222

$$223 \quad \rho_{LTE} = 2(\sqrt{S_0} - \sqrt{S_t}) t^{-1} L^{-1.44} e^{-0.078 T} E^{0.86} \quad (5)$$

224

225 Assuming a *G. morhua* to operate with its individual value, the basic rate parameter ρ_{LTE} can be
 226 considered the outcome of a normally distributed stochastic variable.¹⁴ Then, the performance of
 227 two groups of *G. morhua* with regard to gastric evacuation rate can be compared statistically by
 228 use of a *t*-test to the values of this parameter calculated for each *G. morhua* according to equation
 229 (5). Before *t*-tests were performed (SigmaStat version 3.5) the assumptions of normality
 230 (Kolmogorov-Smirnov test) and homogeneous variance of data (Bartlett's test) were tested.
 231 Significance was accepted at $P < 0.05$.

232

233 CV of ρ_{LTE} is constant and does not depend on the value of this parameter¹⁵. According to Sokal &
 234 Rohlf¹⁶, this implies that the variances of two values of $\ln \rho_{LTE}$ are identical, and that a test of
 235 equality of two values of CV is equivalent to *H*-test of equality of the variances of the logarithmic
 236 transforms of ρ_{LTE} . The latter was therefore used here to test for equality of CV obtained from the
 237 different treatments of fish. Significance was accepted at $P < 0.05$.

238

239 **Results**

240 **Short term recovery (two days)**

241 Two days post surgery, evacuation of force-fed sandeel was considerable slower in fish with
 242 surgical implants compared with control fish. The calculated gastric evacuation rate parameter ρ_{LTE}

243 was thus significantly lower ($P < 0.001$) in implanted *G. morhua* compared to the control, being
 244 respectively 4.2×10^{-4} (1.2×10^{-4}) and 9.3×10^{-4} (1.9×10^{-4}) (mean [SD]) (Figure 2). Because the effects
 245 of fish length were accounted for by ρ_{LTE} (eq. 5), while temperature and energy density of the prey
 246 were similar for all animals (Table I), the observed difference in gastric evacuation rates can be
 247 attributed to pure physiological causes. When comparing our data with the value of $\rho_{LTE} = 14.2 \times 10^{-4}$
 248 (1.1×10^{-4}) obtained in the study by Andersen¹ on *G. morhua* feeding voluntarily on *A. tobianus*
 249 (i.e. involving no handling or anaesthesia prior to recovery of the stomach contents) the present
 250 gastric evacuation rates only constituted approximately one third (fish with implants) and two
 251 thirds (control) of this rate (Figure 2), i.e. both being significantly lower ($P < 0.001$). This difference
 252 was also reflected in that on average only 21 and 42% (instrumented and control fish, respectively)
 253 of the meals had been evacuated 25 h post force-feeding, which is inefficient compared to the
 254 prediction¹ that 62% of the meal should be evacuated.
 255 According to Andersen & Beyer¹⁵ the coefficient of variation (CV) of the basic rate parameter ρ_{LTE}
 256 is constant (i.e. independent of the estimated value of this rate parameter). CV should therefore
 257 be used when comparing the *variability* of ρ_{LTE} in the different groups of *G. morhua*. For the two
 258 groups of force-fed *G. morhua*, CVs of 0.21 and 0.29 (controls and operated, respectively) were
 259 significantly higher ($P < 0.002$ and $P < 0.001$, respectively) than the value of 0.08 obtained from *G.*
 260 *morhua* that fed voluntarily¹ (Table II).

261

262 **Prolonged recovery (ten days)**

263 Ten days post surgery, there was no significant difference ($P = 0.878$) between the rate at which
 264 animals with implants evacuated their meal compared to control fish (Figure 2). These estimates
 265 of ρ_{LTE} were however still significantly lower (7.7×10^{-4} [1.9×10^{-4}] for implanted and 7.9×10^{-4}

[2.8×10^{-4}] for controls) than the value of 14.2×10^{-4} (1.1×10^{-4}) obtained from *G. morhua* feeding voluntarily on *Ammodytes spp.* ($P < 0.001$).¹ Again, as described above for *G. morhua* 2 days post recovery, the coefficients of variation in both of our groups of fish were significantly higher ($P < 0.001$) being 0.24 in surgically implanted fish and 0.35 in the control group (Table II) compared to the value of 0.08 obtained from *G. morhua* that fed voluntarily. In contrast to the improved evacuation ability of the surgically implanted *G. morhua* following prolonged recovery, the percentage of food that had been evacuated by control *G. morhua* at c. 23h post force-feeding (36%) was comparable both to the week earlier (43% c. 25 h post force-feeding after two days of recovery) and to the value for the surgically instrumented fish (36% c. 23 h post force-feeding).

275

Discussion The present study demonstrates that following two days of post-surgical recovery after introduction of a dummy implant into the body cavity, *G. morhua* is not capable of evacuating a meal at a rate similar to non-operated fish. By using ρ_{LTE} (eq. 5) to compare gastric performance between groups of fish we have accounted for the effects of fish length, while temperature and energy density of the prey were similar for all animals (Table I). Our results thus suggest that the observed difference in GER can be attributed to pure physiological causes derived either from surgery, post-surgical stress and/or the presence of the implant. But following ten days of recovery the suppressing effects on gastric performance caused by surgical implantation was gone. Thus at this stage the presence of neither the implant *per se* nor the surgery was limiting the GER. Reduced post-surgical gastric performance has been reported previously. Twenty-four to thirty-six hr subsequent to invasive surgery sea bass *Dicentrarchus labrax* L. showed a significantly higher gastric evacuation time (GET) [the relationship between GET and GER can be deduced from equation (3)] compared to controls, with stomach contents still above 60%

289 of the initial 24 hr post force-feeding, whereas control animals at this time had less than one third
290 of the meal remaining in the stomach.¹⁷ This is thus in accordance with our results showing that
291 GER had not stabilized in *G morhua* two days post surgery.

292

293 A factor that may affect the recovery and even long term function is the effect on buoyancy. A fish
294 with an implant has to counteract the downward force exerted by the added mass which can be
295 done by secretion of gasses into the swimbladder.^{18, 19} With the present implant in the body
296 cavity, a fish, irrespectively of its size, would have to increase its swimbladder volume (5% of the
297 total volume in *G. morhua*¹⁸) with 14.2 cm³ of air to become neutrally buoyant. In the smallest *G.*
298 *morhua* this implies an almost doubling of swimbladder volume, which may approach the limit for
299 their capabilities.²⁰ Swimbladder adjustment is a low cost solution but is slow (up to a day or
300 two)^{21, 22} and meanwhile compensation must occur through active swimming which is
301 energetically expensive.^{19, 23} Such excess energy use will reduce the aerobic scope available for
302 other processes²⁴ and this may be part of the explanation for the lower gastric performance
303 observed two days post surgery. Furthermore, active swimming may result in blood being shunted
304 away from the stomach region to prioritize oxygen delivery to the working muscle.^{25, 26}

305

306 The use of biotelemetry devices allows experimenters to provide their animals with long
307 recovery periods to facilitate complete recovery from instrumentation procedures. Nonetheless,
308 for biotelemetry systems to hold their true/full potential it is imperative that the fish is capable of
309 dealing with not only the effects arising from anaesthesia, handling and surgical intervention but
310 also any potential disadvantages related to carrying the entire recording/transmitting unit. Taken
311 together, our results show that a potential for returning to pre-surgical levels indeed exists, as GER

312 was stabilized 10 days post surgery in fish carrying the implant in the body cavity. Thus the
313 presence of the implant *per se* did not seem to affect the average gastric performance at this
314 point. The fish in the present study weighed between 290 and 815g resulting in transmitter:fish
315 mass ratios from 2.3 to 6.5%. It has been suggested that the mass of telemetry tags should not
316 exceed 2% of body mass^{24, 27}. Several experiments (including experiments on cod) using tags larger
317 than this have however found no significant effect on the swimming performance^{29, 30}. We found
318 no correlation between GER and body mass indicating that cod can recover from carrying tags that
319 represent up to 6.5% of the body mass. Along the same lines results from salmonids on recovery
320 of swimming performance following surgical implantation of telemetry devices have shown that
321 Juvenile Chinook salmon *Oncorhynchus tshawytscha* (Walbaum) had a significantly lower critical
322 swimming speed 1 day post surgery compared to controls, whereas full recovery was
323 accomplished after 21 days (in-between evaluations are lacking);²¹ Although full recovery was not
324 established, the swimming performance of tagged juvenile *Salmo salar* L. had improved 7 days
325 post tagging at termination of the experiment.³⁰ Suggestively the fish may either have recovered
326 in the long term, or the tags, representing on average 8.5% of the fishmass, may have been too
327 heavy or have reduced the mobility of the fish.

328

329 When comparing the variability in gastric performance between different groups of *G. morhua* the
330 coefficient of variation, CV, of the basic rate parameter ρ_{LTE} should be used (for further details on
331 this see Materials and methods). The value 0.080 of CV estimated from the voluntarily feeding fish
332 by Andersen¹ (Table II) compared well with the estimate of 0.098 obtained from a variety of
333 predatory gadoids and their fish prey.¹⁵ This variation probably reflects the inter-individual
334 variation in gastric performance. Substantially higher values of CV were obtained from both

groups of force-fed *G. morhua*, which may then be explained by an additional variability due to the suppressing effects of handling, anaesthetization and force-feeding on GER to which the individual fish responds differently. An alike large variability has been observed in *O. mykiss*; the lag phase from force feeding until the stomach started to empty varied between zero and 5h.³¹ As anticipated, for the present *G. morhua* this furthermore resulted in on average 30% lower values of ρ_{LTE} from control fish compared to the voluntarily feeding *G. morhua* in the study by Andersen¹. Studies on cod using intragastric transmitters have shown that if the fish voluntarily ingest the baited transmitter (in this case a transmitter wrapped in a fillet of herring) high food consumption rates are maintained in the days subsequent to tagging, in contrast to fish tagged by forced insertion (involving handling and anaesthesia) where food intake was notably lower for up to 15 days post tagging.³² This indicate that appetite prevails despite the presence of a transmitter as long as handling and anaesthesia is avoided.

To omit interference from anaesthesia and handling in future studies, one important unanswered question to investigate is thus how long it takes for instrumented fish to commerce voluntarily feeding following the surgical procedure.

Although the primary intention of this study was not to focus on wound healing, this is an important matter, not only for ethical considerations but also because biotelemetric methods enable long-term measurements where open incisions may facilitate internal infections and/or cause changed behavior and performance thus inflicting data invalidity. Two days post surgery the surgically implanted fish had no or only slight signs of inflammatory reaction around the incisions. The exception was two fish, one in which the larger wound had opened, and another where it gapped at one end. These animals were instantly euthanized and data omitted from the analysis.

358 Ten days post surgery an inflammatory response (redness and slight swelling) was noted around
359 all the larger incision made alongside the basibranchiale bone. These observations are in
360 accordance with a previous study in which *G. morhua* (kept at comparable temperatures of 9.5-
361 14° C) were surgically implanted with dummy transmitters into the body cavity via incision along
362 linea alba.³³ In these animals inflammatory responses begun 5-7 days post surgery and subsided
363 for 4-8 days with complete wound healing after a total of 24-34 days. Obviously, the time course
364 of, and how to secure proper wound healing following surgical implantation demands further
365 attention.

366 In summary, two days of post-surgical recovery will not stabilize GER in *G. morhua*, but ten days
367 will, when using a standard force feeding protocol. The results indicate that at ten days post
368 surgery the presence of a dual channel dummy telemetric implant *per se* did not affect GER but
369 that the effects observed 2 days after instrumentation are due to surgery, post-surgical stress
370 and/or the presence of the implant. Biotelemetry has the welfare advantages of allowing long
371 recovery periods and avoiding the unnecessary stress arising from handling and confinement,
372 altogether improving quality of data and we believe our results to leave a promising future for
373 implementation of fully implantable biotelemetry systems in fish.

374

375 **References**

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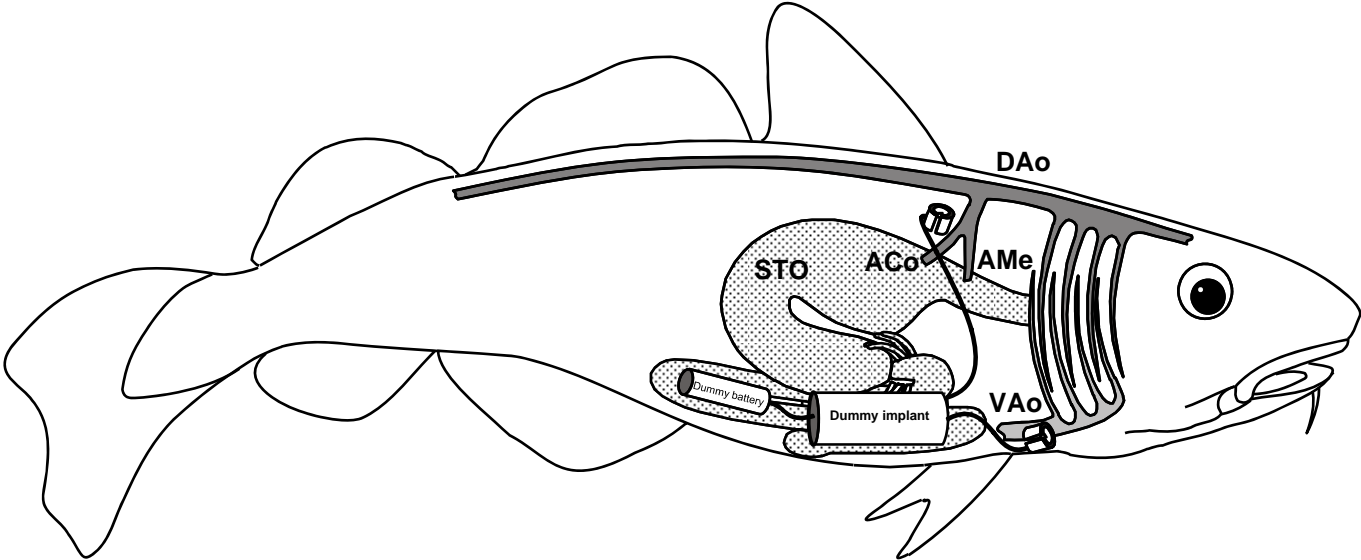
493 Figure 1.

494 Schematic drawing of a *Gadus morhua* showing the placement of the dummy implant and battery
495 (both drawn to scale) in the abdominal cavity and the flow probes belonging to it. The
496 celiacomesenteric artery branches off the dorsal aorta (DAo) and subsequently divides into the
497 celiac (ACo) and the mesenteric artery (AMe). VAo, ventral aorta; STO, stomach

498

499 Figure 2.

500 Comparison of the basic rate parameter ρ (ρ_{LTE}) two and ten days post surgery for force-fed
501 surgically implanted (hatched, n=9) and control (grey, n=8) *Gadus morhua* and with estimates
502 from non-operated voluntarily feeding conspecifics (white).¹ Data are presented as mean (SD), *
503 indicates a significant difference ($P<0.05$) between the force-fed groups two days post surgery, †
504 indicate significant difference ($P<0.05$) between non-operated voluntarily feeding fish and all
505 force-feeding trials (i.e. both two and ten days post surgery).



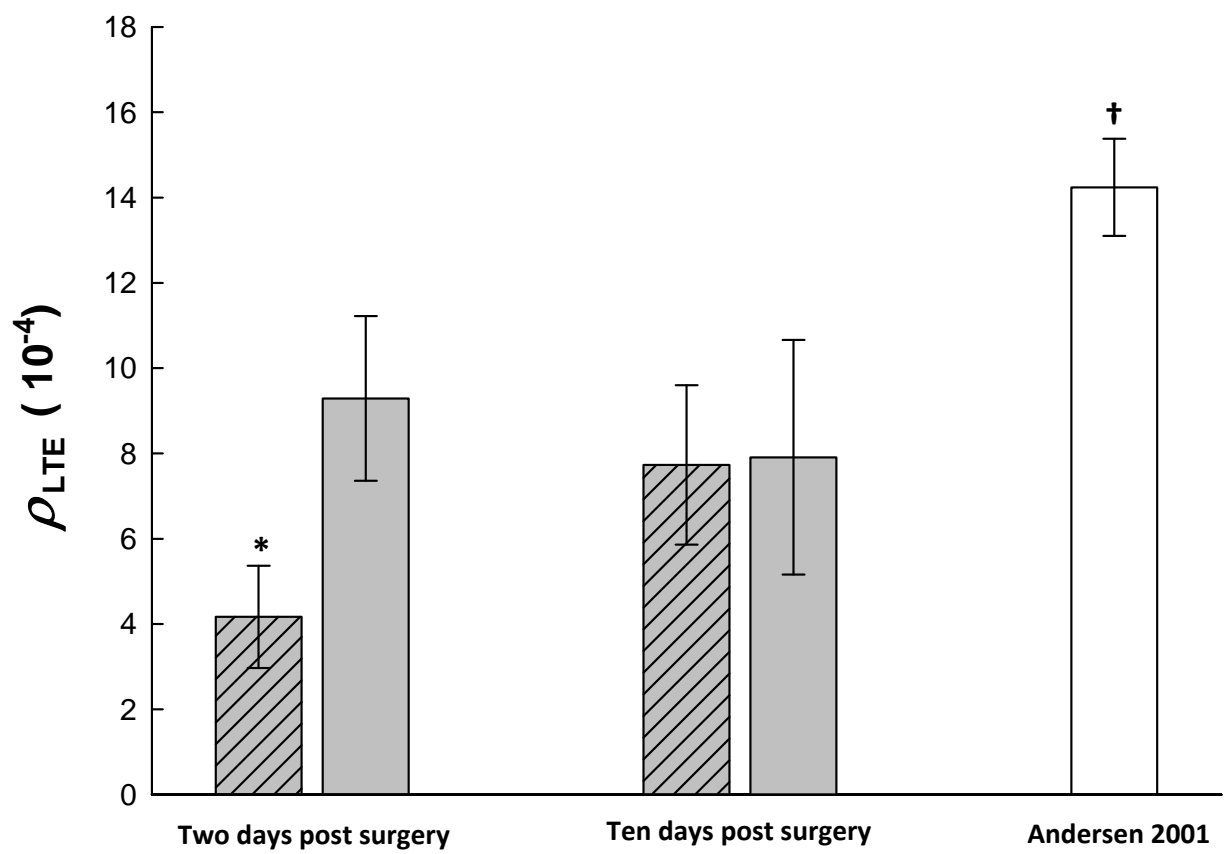


Table I. Basic data on gastric evacuation experiments (mean [SD]) on *Gadus morhua*. All experiments were carried out at 9-10 °C and all meals consisted of a single prey.

Group and experiment	Predator <i>Gadus morhua</i>			Prey <i>Ammodytes marinus</i>			Observations (<i>n</i>)
	<i>L</i> (cm)	Body mass (g)	Time <i>t</i> (h) for recovery of force- fed meal	Original body mass (g)	Recovered body mass at time <i>t</i>	Energy <i>E</i> (kJ g ⁻¹)	
Surgically implanted fish two days post surgery	38.7 (3.2)	550 (144)	25.3 (0.5)	12.9 (1.3)	10.1 (0.8)	6.83	9
Control fish two days post surgery	37.1 (2.7)	518 (155)	25.2 (1.4)	12.3 (1.8)	7.0 (0.9)	6.83	8
Surgically implanted fish ten days post surgery		545 (146)	23.6 (0.8)	12.9 (1.8)	8.2 (1.3)	6.83	9
Control fish ten days post surgery		503 (151)	23.5 (1.3)	11.5 (1.4)	7.2 (0.9)	6.83	8

Table II. Coefficient of variation (CV) of the basic rate parameter ρ_{LTE} obtained from *Gadus morhua* fed an *Ammodytes marinus*. Different letters indicate a significant difference.

	Surgically implanted <i>G. morhua</i> (<i>n</i> =9)	Control <i>G. morhua</i> (<i>n</i> =8)	<i>G. morhua</i> feeding voluntarily on <i>Ammodytes tobianus</i> (<i>n</i> =20) (from Andersen ¹)
Two days post surgery	0.287 ^a	0.207 ^a	0.080 ^b
Ten days post surgery	0.242 ^a	0.348 ^a	